Long-term protective effect of surface sealants against erosive wear by intrinsic and extrinsic acids

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Abstract: OBJECTIVE: To test sealants to prevent erosive tooth wear caused by extrinsic and intrinsic acids under long-term exposition. METHODS: 144 bovine enamel samples were randomly allocated to twelve groups (1-12). Samples of groups 1, 5 and 9 remained unsealed (positive controls), 2, 6 and 10 were sealed with Silicon Seal Nano Mix and 3, 7 and 11 with SealProtect. Groups 4, 8 and 12 were sealed with flowable composite (negative controls). Groups 1-4 were immersed in artificial saliva, 5-8 in hydrochloric acid and groups 9-12 in citric acid for 28 days, respectively. After 1, 2, 4, 7, 11, 14, 21 and 28 days, solutions were renewed and enamel wear was quantified by assignation of (32)P in the solutions. RESULTS: In all immersion solutions, lowest mineral loss was observed for the negative controls whilst highest loss was observed for unsealed positive controls. In artificial saliva and citric acid, the loss from samples sealed with SealProtect was not significantly different compared with negative controls whilst loss in groups sealed with Silicon Seal Nano Mix was significantly higher. In hydrochloric acid, loss from samples sealed with SealProtect was not different compared with that of negative controls up to 4 days. Except day 1, the mineral loss in the SealProtect group was significantly lower compared with that of the Silicon Seal Nano Mix group. CONCLUSION: The tested resin based surface sealant is able to significantly reduce the erosive demineralisation of enamel caused by hydrochloric and citric acid even under long-term exposition.

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Long-term protective effect of surface sealants against erosive wear by intrinsic and extrinsic acids

Running title: Erosion protection by sealants

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Abstract:

Objective: To test sealants to prevent erosive tooth wear caused by extrinsic and intrinsic acids under long-term exposition.

Methods: 144 bovine enamel samples were randomly allocated to twelve groups (1-12). Samples of groups 1, 5 and 9 remained unsealed (positive controls), 2, 6 and 10 were sealed with Silicon Seal Nano Mix and 3, 7 and 11 with Seal&Protect. Groups 4, 8 and 12 were sealed with flowable composite (negative controls).

Groups 1–4 were immersed in artificial saliva, 5–8 in hydrochloric acid and groups 9–12 in citric acid for 28 days, respectively. After 1, 2, 4, 7, 11, 14, 21 and 28 days, solutions were renewed and enamel wear was quantified by assignation of ^{32}P in the solutions.

Results: In all immersion solutions, lowest mineral loss was observed for the negative controls while highest loss was observed for unsealed positive controls.

In artificial saliva and citric acid, the loss from samples sealed with Seal&Protect was not significantly different compared with negative controls while loss in groups sealed with Silicon Seal Nano Mix was significantly higher.

In hydrochloric acid, loss from samples sealed with Seal&Protect was not different compared with that of negative controls up to 4 days. Except day 1, the mineral loss in the Seal&Protect group was significantly lower compared with that of the Silicon Seal Nano Mix group.

Conclusion: The tested resin based surface sealant is able to significantly reduce the erosive demineralisation of enamel caused by hydrochloric and citric acid even under long-term exposition.

Introduction:

Over the last decades, the dental hard tissue loss due to caries has declined in industrialised countries [1,2]. Due to this decline, other reasons for dental hard tissue loss have entered into the focus of dental research, such as dental erosion [3].

Erosion is defined as dental hard tissue loss due to contact of the substrate with acids or chelators in the absence of bacteria [4]. Erosion can be classified depending on the origin of the causing acids, to intrinsic or extrinsic factors [5]. The only intrinsic source for acids is the gastric juice [6] mainly composed of hydrochloric acid [7]. Extrinsic origins of acids are mostly acidic food and beverages [5,8,9], some types of medication, such as acetylsalicylic acid [10] or acidic fumes in chemical or galvanic factories [11-13].

To prevent erosive tooth wear or the associated dental hard tissue softening [14], use of topically applied fluoride formulation is recommended [15]. Already in 1977, Davis and Winter [16] reported that the use of fluoridated tooth pastes could reduce erosive tooth wear when used before an erosive challenge occurs.

The preventive approaches are mostly based on the use of fluoride compounds such as sodium fluoride [17,18], amine fluoride [19] or monofluorophosphate [20]. Beside this traditional approach to prevent erosiv and erosiv/abrasive tooth wear by use of fluoride formulation, different complexes of metal cations (e.g. stannous ions) [21], the use of CO_2 lasers [22] or addition of nano-sized hydroxyapatite [23] and calcium [24] to erosive drinks have been used to find new and more effective formulations and approaches to prevent erosive/abrasive tooth wear. The use of fluorides not only has a protective effect against erosion but also, and maybe more importantly, against caries [25].

Many of the preventive measures concerning erosion prevention depend on patients' compliance, so the chance that the measures fail to prevent erosive tooth wear is still

high [19]. Due to this finding, a more patient independent approach seems to be desirable. Furthermore, a recent study by Austin et al. (2011) [26] showed that the precipitates from the application of Na- and SnF_2-solutions were not able to provide protection against gastric erosion.

In 2003, Schmidlin et al. [27] proposed a sealing of smooth enamel surfaces (inter-proximal) with an adhesive monomer patch for caries prevention. In this study, enamel was also sealed with an unfilled resin (Helibond) which showed promising results although not achieving the preventive effect of the adhesive monomer patch. The adhesive patch and the unfilled resin sealing protected the enamel by formation of a mechanical barrier, preventing the caries inducing lactic acid reaching the enamel underneath. Also for the prevention of erosive tooth wear such a sealing of the enamel has been proposed [28]. Azzopardi et al. (2004) [29] showed a significant protective effect of a resin based surface sealant (Seal&Protect) against erosive tooth wear under *in situ* conditions.

Taking in consideration these findings, the aim of the present study was to test different (by their basic chemical composition) surface sealants to prevent erosive tooth wear caused by extrinsic and intrinsic acids. The hypothesis of the present study was that the enamel is protected by the sealants irrespective of their composition and the acids used.

Materials and Methods:

Sample preparation

For the study, 144 freshly extracted bovine lower incisors were sectioned at the cementum-enamel junction with a water-cooled diamond disc. From the buccal surface of each crown, one sample was prepared by use of a water-cooled diamond trephine mill with an inner diameter of 5 mm. After preparation, the samples were

thinned from the dentine side using water-cooled carborundum paper (waterproof silicon carbide paper, Struers, Erkrath, Germany) until a thickness of approximately 2 mm was achieved. After this thinning, the enamel samples were checked for defects or cracks by transillumination. The samples were irradiated at the Atominstitut der Österreichischen Universitäten (Vienna, Austria) with an exposure time of 85 min and a neutron flow of 0.17x 10^{13} neutrons/cm^2 s to form radioactive ^{32}P.

After a storage time of one week, the samples were sealed at their circumference and the backside with a bonding resin and a flowable composite. For this sealing, the specimens were etched with 37% phosphoric acid for 60 s (Etching Gel, ORBIS DENTAL, Münster, Germany), rinsed with distilled water and dried with oil free air. After drying, the light curing bonding resin Heliobond (Ivoclar Vivadent GmbH, Ellwangen, Germany) was applied for 20 s, blown to a thin layer and light cured for 60 s. For the final sealing, the flowable composite (Orbi-Flow, ORBIS DENTAL, Münster, Germany) was applied and again light cured for 60 s. Finally, samples were checked with a stereomicroscope at a magnification of 40x for continuous sealing of the samples circumference and backside and that the natural enamel surface was free of sealing materials.

The samples were randomly allocated to twelve experimental groups (1 - 12).

Surface sealing procedure

Before performing the surface sealing procedure, all samples of groups 1 - 4 were immersed in artificial saliva. Samples of groups 5 – 8 were immersed in hydrochloric acid (pH 2.3) and the samples of the groups 9 – 12 in citric acid (pH 2.3) for 5 min respectively resulting in a demineralised surface, simulating previously erosion affected surfaces before sealing.

The enamel surfaces of samples of groups 1, 5 and 9 remained unsealed and served as positive controls.

The samples of groups 2, 6 and 10 were sealed with Silicon Seal Nano Mix (S&C Polymer GmbH, Elmshorn, Germany), which was applied on the dried enamel surface in a thin layer and dried for 1 min. This procedure was performed twice according to manufactures instructions.

Samples of groups 3, 7 and 11 were sealed with Seal&Protect (DENTSPLY DETREY GmbH, Konstanz, Germany) according to manufactures instructions. The sealant was applied on the dry enamel surface and left undisturbed for 20 s. After these 20 s the remaining solvent was removed with a blow of an air syringe. Now the sealant was light cured for 10 s. A second layer of sealant was applied, the solvent was removed again with air syringe and again light cured for 10 s.

Chemical composition oft he two surface sealants is given in table 1.

The enamel surface of the samples of the remaining groups 4, 8 and 12 were sealed with the flowable composite as described under sample preparation and served as negative controls.

Immersion in artificial saliva, hydrochloric acid and citric acid

The samples of groups 1 – 4 were immersed in artificial saliva. The artificial saliva was prepared following the composition given by Klimek et al. (1982) [30]. Samples of groups 5 – 8 were immersed in hydrochloric acid (pH 2.3), while the samples of the remaining groups 9 – 12 were immersed in citric acid (pH 2.3).

Immersion was performed at 37 °C under constant motion in 15 ml per sample of the respective solution for a total of 28 days. After 1, 2, 4, 7, 11, 14, 21 and 28 days, the respective immersion solution was renewed. A short overview of the respective surface sealing and immersion solution in the different groups is given in table 2.

Radiochemical analysis

The enamel wear (µg) was quantified by assignation of ^{32}P in the collected solutions by determining the Cherenkow radiation and comparing this radiation with the radiation of known amounts of apatite. The respective laboratory procedure has been described in detail by Schmidlin et al. (2005) [31].

Scanning electron microscopy

To determine whether the surfaces were visibly covered by the sealant or if there were any wetting problems (unprotected surfaces), two additional samples were prepared for each group and scanning electron microscopy pictures were taken using a Supra 50 VP Scanning Electron Microscope (Carl Zeiss NTS, Oberkochen, Germany). SEM pictures were captured at a magnification of 1000x and an acceleration voltage of 5 kV.

Data presentation and analysis

For data presentation of enamel wear, the mean value and standard deviation of enamel in each solution fraction was calculated and presented as cumulative loss of mineralized tissue in micrograms. Within the same immersion solution (artificial saliva, hydrochloric acid and citric acid) the enamel wear after respective days was compared between groups using ANOVA and Scheffe`s post hoc tests. The level for statistical significance was set at $p = 0.05$.

Results:

The cumulative amount of apatite released into the respective immersion solution after respective immersion days (1 day – 28 days) for the different surface sealants is given in Table 3.

Artificial saliva immersion

At all days, significantly highest mineral loss was observed for unsealed samples (positive control), while significantly lowest mineral loss was observed for samples sealed with Heliobond and flowable composite (negative control). Samples sealed with Seal&Protect showed higher mineral loss compared with samples sealed with Heliobond and flowable composite again at all days, although this finding was not significant ($p > 0.05$, respectively). The samples treated with Silicon Seal Nano Mix revealed a significantly lower mineral loss compared with unsealed samples, but a significantly higher loss when compared with samples sealed with Seal&Protect ($p = 0.001$, respectively).

Hydrochloric acid immersion

Lowest mineral loss was observed for samples sealed with Heliobond and flowable composite at all days, however at days 1, 2 and 4 the mineral loss in the Seal&Protect group was not significantly higher ($p > 0.05$, respectively). The unsealed positive control group showed the significantly highest mineral loss over all days, but at days 1, 21 and 28 the mineral loss of samples sealed with Silicon Seal Nano Mix was not significantly lower ($p > 0.05$, respectively). At all days, the mineral loss of the Seal&Protect group was significantly lower compared with the Silicon Seal Nano Mix group, except at day 1.

Citric acid immersion

At all days, significant lowest mineral loss was observed for samples sealed with Heliobond and flowable composite (negative control) while the unsealed samples (positive control) showed the significantly highest mineral loss. The Silicon Seal Nano

Mix group revealed a significantly lower mineral loss compared with unsealed samples. Compared with samples sealed with Seal&Protect, the mineral loss of samples sealed with Silicon Seal Nano Mix was higher ($p = 0.001$, respectively) each day. Over the whole immersion time, the amounts of minerals lost from samples sealed with Seal&Protect were not significantly different compared with the amounts from samples sealed with Heliobond and flowable composite ($p > 0.05$, respectively).

Scanning electron microscopy

SEM pictures of all groups after application of the respective solutions are given in Figure 1.

Samples from groups 5 and 9 (immersion in hydrochloric and citric acid, no sealant) showed distinctly etched surface morphologies. Surfaces of samples treated with the Silicon Seal Nano Mix (groups 2, 6 and 10) showed a surface with isolated spherical coverings, which might be interpreted as the nano-fluorapatite particles. In contrast, the surface of samples treated with Seal&Protect showed a smooth appearance with no visible areas of unprotected enamel. The appearance of the samples treated with Heliobond and flowable composite was rough. This rough surface could be identified at higher magnification (5000x) as a compact packing of filler particles.

Discussion:

For the present study, enamel samples were prepared from bovine lower incisors. The chemical composition and distribution of minerals of bovine and human enamel are comparable as shown in different previous studies [32-34]. An advantage of using bovine teeth is that they are easy to obtain and that usually more than two, sometimes up to six, teeth can be harvested from one animal, while this number of teeth can rarely be gained from one human subject [35]. In addition, bovine teeth used

for studies mostly stem from cattle having grown in a comparable environment with similar forage and that they do not have a history of caries and/or fluoridation measures as human teeth have, which might influence erosive demineralization or adhesion of applied surface sealants. Bovine enamel has been used in numerous studies investigating adhesion of dental adhesives [36-38] and sealing properties of surface sealants [31,39,40]. A resent study by Almeida et al. (2009) showed no significant difference between human and bovine substrates concerning microleakage of adhesively fixed restorations in enamel [41].

The samples of the present study have been immersed in artificial saliva, hydrochloric or citric acid before sealing. It might be imaginable that this pre-immersion might influence the etching and the sealing potential of the materials used due to a lower mineral contend or a mechanical less stable surface of the pre-eroded enamel. However, in the clinical situation such a pre-erosion could not be excluded, as the enamel might have been in contact with extrinsic or intrinsic acids before a sealing procedure might be performed.

In the present study, the ability to prevent erosive tooth wear caused by extrinsic and intrinsic acids by the use of surface sealants was tested by measuring the amount of enamel minerals dissolved in the used liquids (acids or artificial saliva). The amount of enamel was quantified by assignation of ^{32}P in the collected solutions by determining the Cherenkow radiation and comparing this radiation with the radiation of known amounts of apatite. Different other studies concerning prevention of erosive dental hard tissue loss have also measured the amount of certain apatite minerals in the demineralisation solution by chemical analysis of minerals dissolved in the used erosive agent by Arsenazo III procedure [42,43], atomic absorption spectroscopy [44] or by colorimetric methods [45,46]. As one of the used surface sealants (Silicon Seal Nano Mix) contains apatite, it was not feasible to detect certain apatite minerals in the used

solutions since the above listed methods are not able to differentiate between minerals dissolved from the sealed enamel or from the used surface sealant. Further limitation of the above-mentioned methods is that they need specific pH values of the test solutions (colorimetric methods) or are not applicable for all acids (e.g. calcium complexes formed with citric acid impair correct measurement) [47]. Due to these reasons, the amount of enamel dissolved was measured by the radioactive ^{32}P method. This method is capable to determine the apatite loss solely from the enamel as only the sealed enamel contains radioactive ^{32}P but not the used apatite containing sealant and is not affected by different acids or pH values.

Limitation of the present study might be that no storage in remineralization solution such as artificial saliva was performed to simulate the clinical situation, as it has been done in other studies concerning prevention of erosive tooth wear [17,46]. The reason for this was that the present study wanted to simulate the worst-case-scenario. Furthermore, the use of whole human saliva would have lead to the formulation of an acquired pellicle. For this pellicle it is known that it provides protection to the underlying enamel surface against erosive destruction caused by short-term action of citric acid [48]. Using a remineralization solution or human saliva might result in lower amounts of apatite dissolved in the same time periods but should not fundamentally change the findings of the present study.

In the present study, the erosive attack has been performed for a total of 28 days while other recent studies [18,26,46,49] investigating the prevention of erosive tooth wear often used much shorter time periods for the erosive attack [few minutes (6 – 18 min)[26] up to some hours (10 h)[18]]. To simulate the erosive attack, hydrochloric and citric acid has been used, although the dietary substances and intrinsic regurgitating agents are different by their composition. Hydrochloric and citric acid has been used as they represent the main acidic compounds found in acidic foodstuff and

beverages [5] as well in the regurgitated stomach content [7]. Furthermore, these pure acids has been used in numerous other studies simulating erosive attacks [21,26,46,50].

The hypothesis of the present study that there is no difference in the protection against erosion by the two different surface sealants has to be rejected. The resin based sealant Seal&Protect showed very promising results (not significantly different compared to the negative controls in artificial saliva and citric acid) for the whole experimental duration while the Silicon Seal Nano Mix with nano apatite particles showed, especially when hydrochloric acid was used, no statistically significant difference when compared with the unsealed positive controls. Differences in the performance of the sealants when using different acids might be attributed to differences in the chemical constitution of the acids (hydrochloric acid as strong monovalent acid and citric acid as weak polyvalent acid).

The findings that a coating forming sealant (Seal&Protect) shows good protective effect against acidic demineralisation of enamel is in accordance with the findings by Schmidlin et al. (2005) [31] showing a good protective effect when sealing smooth enamel surfaces with two layers of an enamel bonding agent.

The Seal&Protect of the present study showed a superior protective effect when compared with results of a recent study [43] using an anti erosive mouth rinse, containing a combination of $SnCl_2$, AmF and NaF. Seal&Protect provides the same protective effect like a sealing with bonding resin and a flowable composite (negative control) up to 4 days in HCl (pH 2.3) while the anti erosive mouth rinse containing $SnCl_2$, AmF and NaF only reduced the enamel erosion by HCl (pH 2.6) up to 6 min. Even when taking in consideration that the mouth rinse might be used more than once a day, the protective effect of the here used resin based surface sealant last longer and has the advantage that its protective effect is independent of the compliance of the patient.

Erosive tooth wear caused by contact of hydrochloric acid with dental hard tissues is strongly associated with reflux of gastric juice into the oral cavity [51]. 1996, Bartlett et al. [52] found a drop of the oral pH below 5.5 during 0.3% and below pH 6 for 4.4% of the total time during 24-hour pH telemetry in gastro-oesophageal reflux patients, which means an erosion time between 4.3 and 60 min per day, respectively. Taking into consideration even the worse finding that an erosive pH occurs for 60 min per day, it might be calculated that the here used Seal&Protect surface sealant provides a protection for 96 days (four days = 96 h sealing by Seal&Protect divided by 1 h erosive attack per day following the calculations by Bartlett et al. (1996)). However it must be taken in deliberation that in the oral cavity also other circumstances might influence the protective effect like abrasion by tooth brushing or mechanical stressing of the coating by mastication. The same circumstances have to be taken into consideration regarding the protection of a coating forming sealant against erosive tooth wear caused by acidic beverages frequently containing citric acid. However, an *in situ* study by Azzopardi et al. (2004) [29] revealed a significant wear of Seal&Protect due to a 20 days reciprocating erosion/abrasion wear simulation. Despite this finding SEM showed that the coating material remained in place and the authors concluded that the tooth surface is protection against further erosion/abrasion.

By the findings of the present study it might be concluded that a resin based coating forming surface sealant is able to significantly reduce the erosive demineralisation of enamel caused by both hydrochloric and citric acid even under long-term exposition. Further investigation, especially taking in consideration mechanical impacts like tooth brushing and/or mastication, has to be performed to establish more acid resistant enamel sealing agents and sealing protocols.

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Table legends:

Tab. 1: Composition of the two surface sealants (manufactures information).

Tab. 2: Overview of the respective surface sealing (- = none (positive control), SSN = Silicon Seal Nano Mix, S&P = Seal&Protect and H & FC = Heliobond and flowable composite (negative control)) and immersion solution in the different groups.

Tab. 3: Cumulative amounts of apatite in µg (SD) released into the respective immersion solution after respective days (1 day – 28 days) for samples sealed with different surface sealants (- = none, SSN = Silicon Seal Nano Mix, S&P = Seal&Protect and H & FC = Heliobond and flowable composite). Comparisons within the same immersion solution and the same day between different surface sealants that are not significantly different are marked with same capital letters (read vertically in the same immersion solution).

Fig. 1: SEM pictures (1000x) of enamel samples after immersion and treatment with the respective solutions and sealants (- = none, SSN = Silicon Seal Nano Mix, S&P = Seal&Protect and H & FC = Heliobond and flowable composite).

Product	Composition
Seal&Protect (DENTSPLY DETREY GmbH, Konstanz, Germany)	Di- and trimethacrylate resins; PENTA (dipentaerythritol penta acrylate monophosphate); Functionalised amorphous silica; Photoinitiators; Butylated hydroxytoluene; Cetylamine hydrofluoride; Triclosan; Acetone
Silicon Seal Nano Mix (S&C Polymer GmbH, Elmshorn, Germany)	Silicone Polyacrylate resin; Nano-Fluorapatite; Nano-Calciumfluoride; Ethylacetate

Tab. 1: Composition of the two surface sealants (manufactures information).

	Groups											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Surface sealant	-	SSN	S&P	H & FC	-	SSN	S&P	H & FC	-	SSN	S&P	H & FC
Immersion solution	Artificial saliva				Hydrochloric acid				Citric acid			

Tab. 2: Overview of the respective surface sealing (- = none (positive control), SSN = Silicon Seal Nano Mix, S&P = Seal&Protect and H & FC = Heliobond and flowable composite (negative control)) and immersion solution in the different groups.

Immersion solution	Surface sealant	Days							
		1	2	4	7	11	14	21	28
Artificial saliva	-	49.5 (29.4)	73.9 (41.9)	131.1 (70.4)	219.1 (115.0)	337.9 (178.5)	429.0 (227.2)	598.2 (256.4)	809.0 (368.2)
	SSN	29.0 (6.6)	45.4 (10.2)	82.7 (19.3)	137.3 (32.9)	206.1 (50.5)	257.9 (65.8)	374.1 (103.7)	498.2 (145.4)
	S&P	4.5 **A** (3.6)	8.3 **A** (6.1)	15.6 **A** (11.9)	26.0 **A** (20.7)	40.4 **A** (32.4)	50.5 **A** (40.5)	80.0 **A** (56.6)	107.2 **A** (78.1)
	H & FC	0.4 **A** (0.6)	1.0 **A** (1.1)	1.6 **A** (1.8)	2.0 **A** (2.7)	2.8 **A** (4.0)	3.0 **A** (4.4)	4.5 **A** (6.5)	6.2 **A** (9.1)
Hydrochloric acid	-	1478.9 **B** (965.2)	4516.8 (823.8)	8616.9 (729.5)	12853.2 (727.8)	16212.1 (1472.3)	19913.0 (1288.6)	24123.7 **A** (1331.9)	28516.6 **A** (1410.7)
	SSN	959.0 **BC** (832.2)	3569.3 (760.8)	7124.2 (1194.6)	10870.7 (1799.1)	13964.5 (2386.1)	17429.3 (2390.1)	21484.2 **A** (2417.9)	25716.7 **A** (2451.4)
	S&P	286.0 **AC** (244.7)	550.5 **A** (451.6)	1163.7 **A** (913.0)	2051.2 (1566.0)	2896.9 (2080.1)	3772.3 (2662.1)	5616.3 (3707.5)	7577.1 (4856.5)
	H & FC	55.5 **A** (55.3)	101.1 **A** (99.3)	203.5 **A** (200.9)	384.1 (406.8)	629.4 (702.6)	864.4 (964.1)	1342.4 (1407.7)	1746.1 (1835.34)
Citric acid	-	15391.2 (3230.6)	28804.4 (5983.0)	48759.9 (6560.2)	68406.9 (7270.5)	86283.6 (7043.6)	98600.9 (6212.4)	117725.6 (5432.5)	136796.0 (7293.3)
	SSN	7971.7 (1275.6)	14313.7 (2058.5)	24201.3 (3550.6)	36778.4 (5081.0)	48629.8 (8318.9)	57945.8 (9046.9)	76072.0 (9423.4)	94653.0 (10241.5)
	S&P	128.9 **A** (131.6)	277.4 **A** (274.3)	553.5 **A** (536.7)	1036.4 **A** (984.4)	1727.1 **A** (1664.6)	2266.6 **A** (2179.7)	3558.2 **A** (3379.7)	5215.7 **A** (4748.4)
	H & FC	84.5 **A** (105.8)	179.3 **A** (224.1)	366.2 **A** (439.9)	712.6 **A** (814.5)	1185.1 **A** (1317.4)	1529.6 **A** (1661.1)	2344.6 **A** (2505.6)	3396.0 **A** (3566.0)

Tab. 3: Cumulative amounts of apatite in µg (SD) released into the respective immersion solution after respective days (1 day – 28 days) for samples sealed with different surface sealants (- = none, SSN = Silicon Seal Nano Mix, S&P = Seal&Protect and H & FC = Heliobond and flowable composite). Comparisons within the same immersion solution and the same day between different surface sealants that are not significantly different are marked with same capital letters (read vertically in the same immersion solution).

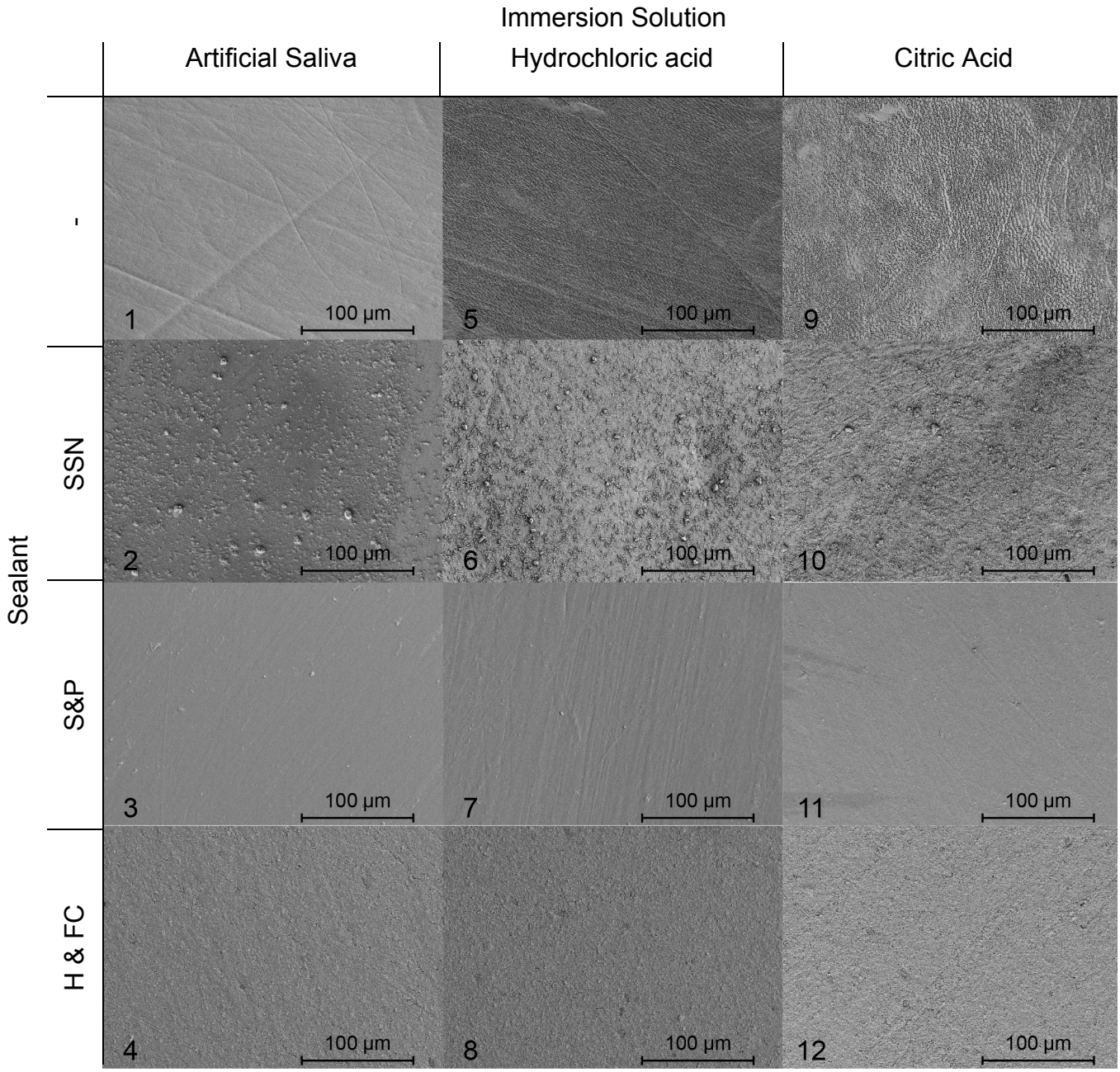
Immersion Solution
Artificial Saliva
Hydrochloric acid
Citric Acid
Sealant
-
SSN
S&P
H & FC
1
2
3
4
5
6
7
8
9
10
11
12
100 µm

Fig. 1: